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Genetic Characterization of the Hemagglutinin of Two Strains of Influenza B Virus Co-Circulated in Taiwan

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Two isolates of influenza B virus were obtained in the spring of 1997. One strain, B/Taiwan/21706/97, was isolated from a patient who had acute tonsillitis. The other, B/Taiwan/3143/97, was isolated from a patient who was diagnosed with meningoencephalitis. This implies that the influenza B viruses not only cause respiratory symptoms but may also cause inflammation of the nervous system. Sequence analysis of the hemagglutinin (HA) gene, HA1 domain, indicated that there were remarkable amino acid changes in the strain B/Taiwan/3143/97 compared to B/Victoria/2/87, B/Yamagata/16/88, and B/Taiwan/7/88. The changes in the positions 116, 200, 238, 242, and 271 were correlated with receptor binding. Furthermore, a potential glycosylation site at position 233 was lost. In total, 30 amino acid changes were noted at positions ranging from 116 to 295. These changes may affect the antigenicity of the virus. Phylogenetic analyses also showed that the B/Taiwan/3143/97 was located in an independent lineage, when compared to the reference strains belonging to B/Victoria/2/87 and B/Yamagata/16/88 lineages. This supports the hypothesis that influenza B viruses with distinct genetic characteristic were co-circulated in Taiwan. ***J. Med. Virol. 59:208–214, 1999.***

KEY WORDS: meningoencephalitis; PCR; nucleotide sequences; phylogenetic tree

INTRODUCTION

Influenza viruses are negative sense, single-stranded RNA viruses with segmented genomes, and on the basis of antigenic differences in nucleoproteins can be divided into three types: A, B, and C [Zuckerman et al., 1993]. All three types of the influenza viruses vary in degrees of morbidity and mortality. Influenza A, for example, is the most virulent virus, widespread in distribution, and characterized by its ability to cause more severe respiratory diseases than influenza B [Yamashita et al., 1988]. In contrast, influenza C causes only mild respiratory infections, mainly in the immunologically compromized or in elderly persons. Among the various viral proteins, the hemagglutinin is the main determinant of virulence and can undergo antigenic variation, which allows the viruses to escape detection of the host's immune system [Wiley and Skehel, 1987].

Two distinct degrees of the antigenic variation occur in influenza A viruses: antigenic drift and antigenic shift [Air et al., 1990]. The antigenic drift in the genes coding for hemagglutinin (HA) and neuraminidase (NA) may cause the amino acid sequences to change, thus altering the antigenic properties and producing a gradual distancing of new strains from the original virus [Yamashita et al., 1988]. The antigenic shift occurs when two viruses infect the same host. Genetic reassortment of the viral genes may produce antigenic, completely distinct, new viruses, and may cause pandemic disease; an example is the 1918 pandemic of in-

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Sequences reported in this article have been deposited in the NCBI database and assigned the accession numbers AF026161 and AF026162.

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TABLE I. Influenza B Virus Strains Used in Analyses of the HA Gene

Virus	Abbreviation	Accession no.	Year of isolation	Reference
B/Lee/1/40	Lee40	K00423	1940	Krystal et al. [1983]
B/Great Lakes/54	GL54	M22947	1954	Yamashita et al. [1988]
B/Maryland/59	ML59	K00424	1959	Krystal et al. [1983]
B/Singapore/64	SG64	M22946	1964	Yamashita et al. [1988]
B/Hongkong/8/73	HK73	M10298	1973	Hovanec et al. [1984]
B/Singapore/222/79	SG79	X00897	1979	Verhoeyen et al. [1983]
B/Oregon/5/80	OR80	K02713	1980	Berton et al. [1984]
B/ENG/222/82	ENG82	X17222	1982	Robertson et al. [1987]
B/USSR/100/83	USSR83	X13552	1983	Air et al. [1988]
B/Idaho/1/86	ID86	M22945	1986	Yamashita et al. [1988]
B/Georgia/1/86	GA86	M22944	1986	Yamashita et al. [1988]
B/Memphis/6/86	MS86	X13551	1986	Air et al. [1988]
B/Ann Arbor/1/86	AA86	M21874	1986	Bootman et al. [1988]
B/Victoria/2/87	VI87	M58428	1987	Rota et al. [1990]
B/Beijing/1/87	BJ87	X53098	1987	Dayan et al. [1990]
B/Yamagata/16/88	YA88	M58419	1988	Rota et al. [1990]
B/Taiwan/7/88	TW88	M58421	1988	Rota et al. [1990]
B/Ohio/10/88	OH88	M58426	1988	Rota et al. [1990]
B/Guangdon/55/89	GD89	M65166	1989	Rota et al. [1992]
B/Hongkong/22/89	HK22/89	M65167	1989	Rota et al. [1992]
B/Hongkong/9/89	HK9/89	M65169	1989	Rota et al. [1992]
B/Victoria/19/89	VI89	M65177	1989	Rota et al. [1992]
B/Bangkok/163/90	BK90	M65165	1990	Rota et al. [1992]
B/Panama/45/90	PN90	M65171	1990	Rota et al. [1992]
B/Paris/329/90	PS90	M65173	1990	Rota et al. [1992]
B/Texas/4/90	TX90	M65175	1990	Rota et al. [1992]
B/Czechoslovakia/69/90	CS90	L76314	1990	Ikonen et al. [1996]
B/Stockholm/10/90	SH90	L76331	1990	Ikonen et al. [1996]
B/Switzerland/5241/90	SW90	L76333	1990	Ikonen et al. [1996]
B/Finland/172/91	FL91	L76316	1991	Ikonen et al. [1996]
B/Khazkov/224/91	KK91	L76322	1991	Ikonen et al. [1996]
B/Leningrad/148/91	LG91	L76325	1991	Ikonen et al. [1996]
B/Finland/268/93	FL93	L76320	1993	Ikonen et al. [1996]
B/Taiwan/21706/97	TW21706	AF026161	1997	This report
B/Taiwan/3143/97	TW3143	AF026162	1997	This report

fluenza A virus. In contrast, no antigenic shift has ever been detected in influenza B viruses. There are also no subtype divisions of the surface antigens in B, as there are in the influenza A viruses. However, analyses of the nucleotide sequence of the HA genes of field isolates of influenza B viruses suggest that antigenic drift also occurs in the viruses [Rota et al., 1990]. Epidemiological data also suggest that a different evolutionary pattern may exist for influenza B virus. Rather than by the prevalence of a single dominant virus, as seen with influenza A virus, influenza B virus epidemiology is characterized by co-circulating lineage at the same period [Yamashita et al., 1988; Rota et al., 1992].

In the spring of 1997, there was an epidemic of influenza B in Taiwan. Two strains of influenza B viruses were isolated at the Veterans General Hospital, Taipei. Both strains have been identified further by the Centers for Disease Control and Prevention (CDC) (Atlanta, GA). One strain designated as B/Taiwan/21706/97 (TW21706/97) was isolated from a patient with acute tonsillitis. However, the other strain, B/Taiwan/3143/97 (TW3143/97), was recovered from a patient who had been diagnosed with meningoencephalitis. This finding would imply that not only does influenza B virus cause respiratory symptoms, but it also may affect the nervous system as well as influenza A virus [Salonen et al., 1997; Ward and DeKoning-Ward, 1995]. Both encephalopathy and encephalitis caused by influenza B virus have been reported [Fujimoto et al., 1998]. For this reason, it would be of interest to verify whether there are any differences between these two strains of influenza B viruses. The results of this experiment indicate that there are critical changes in the globular head of the hemagglutinin domain in B/Taiwan/3143/97. The phylogenetic result also suggests that B/Taiwan/3143/97 emerged from a distinct branch among other well-documented strains of the influenza B viruses.

MATERIALS AND METHODS

Specimen Collection and Virus Growth

Throat swabs were collected from two children, 12 and 9 years of age, at the Department of Pediatrics, Veterans General Hospital, Taipei. They were diagnosed with acute tonsillitis (B/Taiwan/21706/97) and with meningoencephalitis (B/Taiwan/3143/97), respectively. The viruses were grown in Madin-Darby canine kidney (MDCK) cell line. Table I lists the influenza B viruses examined, year of isolation, GenBank accession numbers, and their abbreviations.

TABLE II. Primers Used in This Study

Primers	Sequence	Location
Primer B/17	5′-TTTCTAATATCCACAAAATGA-3′	17–37
Primer B/1140	5′-ACCAGCAATAGCTCCGAAGAACC-3′	1140–1118
Primer B/403	5′-AATCTTCTCAGAGGATATGAA-3′	403–423
Primer B/961	5′-GGCAATCTGCTTCACCAATTAAAGG-3′	961–937
Primer T7	5′-TAATACGACTCACTATAGGGCGA-3′	—[a]
Primer Sp6	5′-ATTTAGGTGACACTATAGAATACT-3′	—

[a]Both primers T7 and Sp6 are universal primers of pGEM-T vector flanking the multiple cloning site.

RNA Extraction

Virion RNA was extracted by using the commercialized RNA isolation reagent, TRI REAGENT™LS (Molecular Research Center, Inc.) as described previously [Chomczynski, 1993]. Briefly, 1 volume (0.25 ml) of the virus-infected cells was mixed with 3 volumes (0.75 ml) of the TRI REAGENT™LS. After the sample was homogenized, 0.2 ml of chloroform was added and the sample was shaken vigorously for 15 seconds and then stored at room temperature for 2 to 3 minutes. The resulting mixture was centrifuged at 12,000g for 15 minutes. The aqueous phase was collected; the viral RNA precipitated by isopropanol and then washed with 70% ethanol. Finally, the purified RNA was dissolved with 9 μl sterilized de-ionized water.

Reverse Transcription and PCR

The cDNA synthesis and PCR amplifications of the coding regions of the HA1 domains of the HA genes were carried out using primers corresponding to nucleotides 17-37 (Primer B/17) and 1140-1118 (Primer B/1140) (Table II) producing a 1.1 kb double stranded DNA fragment. Afterwards, a two-stage amplification was adopted by using nested primers corresponding to nucleotides 403-423 (Primer B/403) and 961-937 (Primer B/961) (Table II), yielding a 562 bp fragment, using the procedure by Robertson et al. [1990]. Briefly, 9 μl of the RNA preparation was mixed with 16 μl of the buffer containing 50 mM KCl, 10 mM Tris-HCl, pH 8.4, 2.5 mM $MgCl_2$, and 0.02 gelatin, 1 mM each of dNTP's, 2 units of RNase inhibitor, 50 pmol of oligonucleotide (Primer 17), and 1 μl of avian myeloblastosis virus reverse transcriptase (AMV RT, RNase H minus, Promega, Madison, WI) (5–10 unit/μl). The mixture was incubated at 42°C for 60 minutes to make cDNA. For PCR, 100 μl of reaction mixture containing amplification buffer (10 mM Tris-HCl, pH 8.3, 50 mM KCl, 2.5 mM $MgCl_2$, and 0.02 gelatin), 20 pmol each of the primers, 1 μl (4 unit) of KlenTaq DNA polymerase (Clontech, Palo Alto, CA), and 25 μl of cDNA solution. The amplification reaction was performed at 94°C for 0.5 minutes, 55°C for 30 seconds, and 72°C for 1 minute for 30 cycles in a DNA thermal cycler (GeneAmp PCR System 2400, Perkin-Elmer, Oak Brook, IL). One tenth of the amplified product was applied on the 1.5 agarose gel and stained with ethidium bromide. The PCR products on an electrophoresis of agarose gel revealed a band of 562 base pairs, which was visible under UV illumination.

Cloning and Sequencing of HA Genes

The PCR-derived dsDNA was ligated into the pGem-T vector (Promega, Madison, WI) and transformed into *Escherichia coli* JM109. The positive clones were selected and cultured in L-broth containing 100 μg/ml ampicillin (Sigma Chemical Co., St. Louis, MO) and were incubated at 37°C overnight. The bacteria were centrifuged at 3,000 rpm for 15 minutes. The pellet was treated with the Wizard™ Minipreps DNA Purification System (Promega, Madison, WI) to extract the plasmid DNA, which was used as a template for automated sequencing on an Applied Biosystem 373A automated DNA sequencer using cycle sequencing dye terminator chemistry (Perkin Elmer, Foster City, CA). T7 and Sp6 primers were used to sequence the HA1 domain of the HA gene. Table II lists the primers used in this study.

DNA and Amino Acid Sequence Analysis

The nucleotide sequences were analyzed by using the computer software, DNAsis, for nucleotides-amino acid translation. The computer software, MEGA®, version 1.01 [Kumar et al., 1993], was used to align the amino acid sequences and to construct the phylogenetic tree from the sequence data.

Nucleotide Sequence Accession Numbers

The nucleotide sequences for the strains in Table I are available from GenBank under their respective accession numbers.

RESULTS

Comparative Analysis of Nucleotide and the HA1 Domain

For comparison, the nucleotide sequences of the HA1 variable regions of the hemagglutinin genes of the B/Taiwan/3143/97 and B/Taiwan/21706/97 were determined and compared with those of the previously sequenced genes of influenza B viruses [Rota et al., 1990].

The nucleotide sequences and deduced amino acid sequences determined in this study are shown in Figures 1 and 2, along with those of the hemagglutinin genes of influenza B virus sequenced previously. The strain, B/Taiwan/21706/97, differed only by 19 nucleotide sequences and the degree of nucleotide sequence homology of the hemagglutinin gene was relatively high (97%). In contrast, the strain, B/Taiwan/3143/97, which was isolated from the meningoencephalitic patient, differed by 45 nucleotide sequences (that in-

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                         410       420       430       440       450       460       470
B/Victoria/2/87    AATCTTCTCAGAGGATACGAACATATCAGGTTATCAACCCATAACGTTATCAACGCAGAAACGGCACC
B/Yamagata/16/88   .................T...A.......A...............................G......
B/Taiwan/7/88      .................T...A.......A...............................G......
B/Taiwan/21706/97  .................T....G..............A...............T.......A......
B/Taiwan/3143/97   .................T...AGA....CA..........CA.........TC........A......

                           480       490       500       510       520       530
B/Victoria/2/87    AGGAGGACCCTACAAAGTTGGAACCTCAGGGTCTTGCCCTAACGTTACCAATGGAAACGGATTCTTCG
B/Yamagata/16/88   ..............G.C.............A...................G.A...............
B/Taiwan/7/88      ..............G.C.............A...........T.......G.A...............
B/Taiwan/21706/97  ...........G....A...................................................
B/Taiwan/3143/97   ...........T...TC.............A.............C.......A...G......T....

                   540       550       560       570       580       590       600
B/Victoria/2/87    CAACAATGGCTTGGGCTGTCCCAAAAAACGACAACAACAAAACAGCAACAAATCCATTAACAGTAGAA
B/Yamagata/16/88   ........................GGG..A..---..A---........G......C...........
B/Taiwan/7/88      ........................GGG..A..---..A---........G......C...........
B/Taiwan/21706/97  ...............................A.....................T........A.....
B/Taiwan/3143/97   ........................GGG..A..---..............G......C...........

                     610       620       630       640       650       660       670
B/Victoria/2/87    GTACCATACATTTGTACAGAAGGAGAAGACCAAATTACTGTTTGGGGGTTCCACTCTGATAGCGAAAC
B/Yamagata/16/88   ..............C...A..................................T......GA.A....
B/Taiwan/7/88      ..................A..................................T.........A....
B/Taiwan/21706/97  ......................................C......................A......
B/Taiwan/3143/97   ......C....C...T.GA...A..............................T.......A.A....

                       680       690       700       710       720       730       740
B/Victoria/2/87    CCAAATGGTAAAACTCTATGGAGACTCAAAGCCTCAGAAGTTCACCTCATCTGCCAATGGAGTAACCA
B/Yamagata/16/88   .......AA.....................T.....A...............................
B/Taiwan/7/88      .......AA...C.................T.....A...............................
B/Taiwan/21706/97  ........C....................................T...........C.....G....
B/Taiwan/3143/97   ..CC...AA...C.................T.....A...............................

                         750       760       770       780       790       800       810
B/Victoria/2/87    CACATTACGTTTCACAGATTGGTGGCTTCCCAAATCAAGCAGAAGACGGAGGGCTACCACAAAGCGGT
B/Yamagata/16/88   .......T.....T..........A.............A............................C
B/Taiwan/7/88      .......T.....T........................A............................C
B/Taiwan/21706/97  .C....................................A.............A...........T...
B/Taiwan/3143/97   .C.....T.....T..C.....C........GG....CA........C............C......C

                           820       830       840       850       860       870
B/Victoria/2/87    AGAATTGTTGTTGATTACATGGTGCAAAAATCTGGAAAAACAGGAACAATTACCTACCAAAGAGGTAT
B/Yamagata/16/88   ..............................C....G..............AGT...T.........G.
B/Taiwan/7/88      ..............................C....G...............GT...T.........G.
B/Taiwan/21706/97  ........................................................T......A....
B/Taiwan/3143/97   ......A...........C...........C....G......C....C...GT..CT.T.........

                   880       890       900       910       920       930       940
B/Victoria/2/87    TTTATTGCCTCAAAAAGTGTGGTGCGCAAGTGGCAGGAGCAAGGTAATAAAAGGGTCCTTGCCTTTAA
B/Yamagata/16/88   ...G...........G....................................................
B/Taiwan/7/88      ...G...........G....................................................
B/Taiwan/21706/97  ......................................................A.............
B/Taiwan/3143/97   ...G........C..G...........G......CA....CCA...C..G.............C....

                     950       960
B/Victoria/2/87    TTGGTGAAGCAGATTGC
B/Yamagata/16/88   .................
B/Taiwan/7/88      .................
B/Taiwan/21706/97  .................
B/Taiwan/3143/97   ........C........
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Fig. 1. Comparison of the partial HA1 nucleotide sequences ranging from 403 to 963 of the influenza B viruses. Dots and dashes indicate nucleotides that are identical to that of the VI 87 and deletions, respectively.

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                 110       120       130       140       150       160       170
B/Victoria/2/87   NLLRGYEHIRLSTHNVINAETAPGGPYKVGTSGSCPNVTNGNGFFATMAWAVPKNDNNKTATNPLTVE
B/Yamagata/16/88  .......N............R......RL..........SR............RDN-K-.........
B/Taiwan/7/88     .......N............R......RL..........SR............RDN-K-.........
B/Taiwan/21706/97 .......R....N.......K.....C.I..........................E.......S..I.
B/Taiwan/3143/97  .......R.T...P...S..K.....FNL........A..RS...........RDN-...........

                   180       190       200       210       220       230       240
B/Victoria/2/87   VPYICTEGEDQITVWGFHSDSETQMVKLYGDSKPQKFTSSANGVTTHYVSQIGGFPNQAEDGGLPQSG
B/Yamagata/16/88  ......K.............DK...K......N....................D....T.........
B/Taiwan/7/88     ......K..............K...KN.....N.........................T.........
B/Taiwan/21706/97 ....................N....A................................T.........
B/Taiwan/3143/97  ..H..SKE............NK.P.KN.....N.................H.....DHT..R...P..

                     250       260       270       280       290
B/Victoria/2/87   RIVVDYMVQKSGKTGTITYQRGILLPQKVWCASGRSKVIKGSLPLIGEADC
B/Yamagata/16/88  ..........P......V....V............................
B/Taiwan/7/88     ..........P......V....V............................
B/Taiwan/21706/97 .....................S.............................
B/Taiwan/3143/97  ..I...L...P...R..VSL......H.......Q.P.LE........P..
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Fig. 2. Comparison of the partial HA1 amino acid sequences from residues 109 to 295 of the influenza B viruses. Dashes indicate amino acid deletions.

cluded a three nucleotide deletion at the position 571-573) from the B/Victoria/2/87, showed that the degree of nucleotide sequence homology of the hemagglutinin gene was comparatively low (92%). This result suggests that a high degree of antigenic drift had occurred among the hemagglutinin gene of the strain, B/Taiwan/3143/97. Because the strain, B/Taiwan/3143/97, showed a striking difference in nucleotide sequence of hemagglutinin gene, two additional clones of plasmids were also examined to confirm the sequence of hemagglutinin gene. The results were identical with the original clone (data not shown). For comparison of the deduced amino acid sequence, the strain, B/Taiwan/21706/97, differed by only 11 amino acids and was genetically closely related to that of the reference strains. In contrast, the deduced amino acid sequence of hemagglutinin of the strain, B/Taiwan/3143/97, also differed by 30 amino acids from the B/Victoria/2/87 and other well-documented strains.

Phylogenetic Analyses

Phylogenetic analyses were undertaken by the software, MEGA® [Kumar et al., 1993] based on Kimura-2 parameter [Kimura, 1980] and neighbor-joining methods [Saitou and Nei, 1987] on the nucleotides (Fig. 3). On the basis of the derived topology, the B/Taiwan/3143/97 strain was classified into a distinct lineage compared with the B/Taiwan/21706/97 strain and the reference strains. In contrast, the B/Taiwan/21706/97 strain was similar to the reference strains. This finding showed the B/Taiwan/3143/97 strain to be very different from others.

DISCUSSION

Although Reye's syndrome, with a noninflammatory encephalopathy may be associated with infection with influenza B virus [Davis et al., 1985; Davis, 1987], little data are available for the association with meningoencephalitis. Although no brain biopsy material was available to support B/Taiwan/3143/97 strain as directly associated with the meningoencephalitis from the 9-year-old patient, the patient showed both Kernig and Brudzinski signs. At the time of the present study, influenza encephalopathy and encephalitis were reported in Japan [Fujimoto et al., 1998]. Both influenza A and B viruses were isolated from children with generalized convulsions. Owing to the geographical proximity of Taiwan and Japan, influenza B viruses with similar properties may have affected both countries simultaneously.

Compared to the DNA sequences of haemagglutinin genes of influenza B from 1940 to 1997, a striking change was also found in the HA domains of the strain, B/Taiwan/3143/97. It has been shown that changes of six amino acids in the global head region of HA1 domain resulted in the recognition of both α2,3 and α2,6 sialyl linkages instead of α2,3 sialyl linkage only [Xu et al., 1996]. Results of the present study show that the changes in positions 116 and 271 are similar to the results of Xu et al. [1996]. Xu et al. [1996] also indicate that although the receptor binding sites of influenza B virus are currently not known, change of amino acids at six positions may be sufficient to change the receptor binding specificity in the virus. In addition, arginines at positions 238, and 259 in B/Taiwan/3143/97 possess the longest aliphatic chain, whereas glycine has only one single hydrogen atom at its side chain at these positions in other strains and also differs from arginine in biochemical properties. Similarly, proline at positions 200 and 242 in B/Taiwan/3143/97 contains a secondary amino group differing from glutamine in other strains, which contains a terminal amide group. These

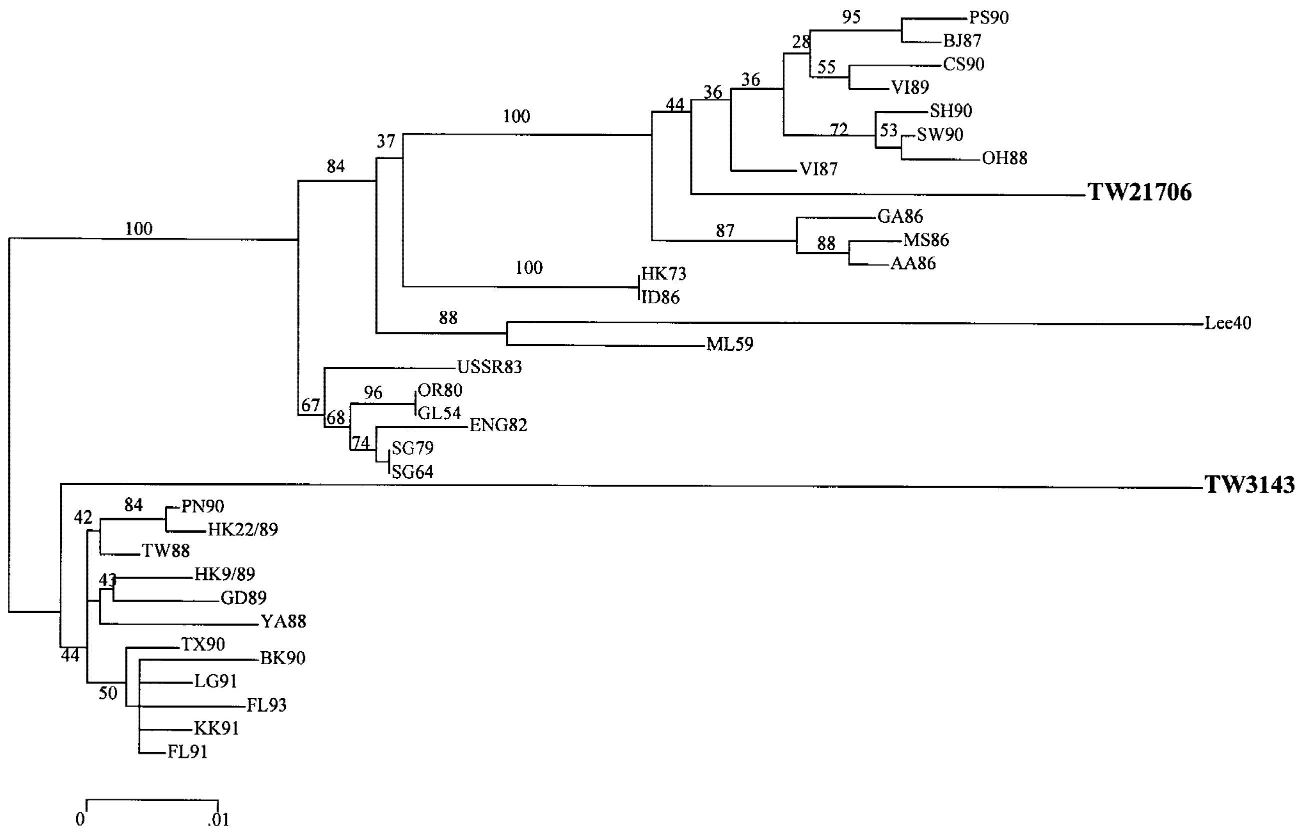

Fig. 3 Phylogenetic relationships among the influenza B viruses. An unrooted neighbor-joining tree of HA1 nucleotide sequences was generated followed by 1,000 replications of bootstrap-resampling. The number at each branch point indicates percentage probability that the resultant topology is correct. The lengths of the horizontal lines are proportional to the nucleotide changes between sequences. Vertical lines separate progeny virus lineages at the point where they branch from a theoretical common ancestor.

differences in biochemical properties may affect receptor-binding affinities [Xu et al., 1996]. In addition, previous work has noted that there is a conservation of some amino acids at the influenza A and B receptor binding sites (i.e., positions 95, 158, 199, 203, and 204). Substantial alterations in the left edge of the influenza B HA receptor-binding sites (235–243) may affect the specificity of the sialyl-galactose linkage recognition, as described in Matrosovich et al. [1993]. In the present study, the mutation sites at the positions 238 (E→R) and 242 (Q→P) may also affect the recognition of the receptor-binding site.

In contrast to the conclusion reached by Xu et al. [1996], Rivera et al. [1995] indicate that amino acids 196-202 are highly surface-accessible. Instead of helical structure, they predict that this region may form an extended surface loop, which does not participate in receptor binding. This loop appears to contribute a major portion of the antigenic properties of influenza B HA since all the major epidemic variants sequenced show changes in this region. The B/Taiwan/3143/97 strain substituted at the positions 197 (S→N) and 200 (Q→P) may thus affect the antigenicity of B/Taiwan/3143/97. The oligosaccharides of human viruses may also promote virus growth by masking antigenic sites, as described by Pyhala et al. [1995]. A glycosylation site at position 233 as noted in this study changed from asparagine to aspartic acid in B/Taiwan/3143/97. This change may alter affect the antigenicity of the viruses and its survival in the hosts.

Phylogenetics examined by neighbor-joining and bootstrap-resampling methods used on the nucleotides was performed on the isolates and on 33 reference strains. According to distances in the phylogenetic tree, the B/Taiwan/3143/97 strain was located in an independent lineage when compared to the reference strains. This implies that such a difference as exhibited by B/Taiwan/3143/97 may be associated with its ability to target the nervous system. The influenza B viruses isolated in different parts of the world since 1987 belong to two distinct evolutionary lineages: the B/Victoria/2/87-like viruses (VI/87 branch) and the B/Yamagata/16/88-like viruses (YA/88 branch) [Kinnunen et al., 1992]. The present finding indicates that B/Taiwan/3143/97 seems to have emerged from the YA/88 branch but was also very different genetically from those virus strains of that branch (Fig. 3). In fact, none of virus strains of the YA/88 branch have been reported in association with neurological symptoms. From this phylogenetic tree, it was also found that B/Taiwan/21706/97, another strain isolated here, was near to the VI/87 lineage. This would suggest that B/Taiwan/

21706/97 is similar to those reference strains. Furthermore, the present study also shows the existence of co-circulation in the influenza B viruses and confirms the finding of Yamashita et al. [1988]. It concluded that B/Taiwan/3143/97 and B/Taiwan/21706/97 existed at the same time but in fact belonged to different lineages.

Finally, antigenic drift as described in the hemagglutinin might have resulted in an increase of virulence of the influenza B virus by the following mechanism: First, change in the conformation of the antigenic sites may help the viruses to evade the host's humoral and cellular immune responses. Second, change in the viral receptor binding sites may broaden the tissue tropism, even difference in clinical appearance. For example, B/Taiwan/3143/97 may move to its receptor binding sites and transfer as common upper respiratory symptoms to neurological involvement. Third, change in the acute phase protein binding sites may resist the opsonization by the acute-phase proteins at early infection (i.e., mannose-binding lectins) [Hartley et al., 1997]. Since acute-phase proteins are several different plasma proteins, they increase in concentration in response to injurious stimuli, including early infection, burns, trauma, neoplasia, etc. Further study on the pathogenesis of influenza-associated meningoencephalitis is currently in progress.

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